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REPORT

MUTATIONS PRODUCED BY ATOMIC BOMB IRRADIATION OF NEUROSPORA CRASSA

APPENDIX NO. 16
TO THE FINAL REPORT

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7 March 1949

MUTATIONS PRODUCED BY ATOMIC BOMB IRRADIATION OF NEUROSPORA CRASSA*

Report of Naval Medical Research Section, Joint Task Force ONE, on Biological Aspects of Atomic Bomb Tests.

APPENDIX No. 16

by

CARROLL E. WAGNER, HMC, USN
Naval Medical Research Institute
Bethesda, Md.

and

G. W. BEADLE
Chairman, Division of Biology
California Institute of Technology
Pasadena, California

Approved:
R. H. DRAEGER
Captain, MC, U. S. Navy
Officer-in-Charge

* This work was conducted at the Kerckhoff Biological Laboratories of the California Institute of Technology, Pasadena, California under Office of Naval Research Contract N6-onr-244-Task Group V.

MUTATIONS PRODUCED BY ATOMIC BOMB IRRADIATION OF NEUROSPORA CRASSA.

Introduction

In the planning of the biological experiments for the Bikini atomic bomb tests it was considered desirable to expose an organism which while readily reactant to ionizing radiation from a genetic viewpoint, would survive the other conditions involved in a prolonged trip to the tropics.

From the work of Beadle and Tatum (1) and Beadle (2) it was known that the red bread mold, Neurospora crassa, was an ideal organism from a genetic standpoint and that mutations were readily produced by ionizing radiation. Further, samples of this organism could be prepared in such a manner so as to survive for indefinite periods of time.

Neurospora crassa is one of the bread molds that grow in tropical and semitropical regions. It produces great masses of brilliant orange asexual spores known as conidia. It is a heterothallic fungus, although the two sexes are not visibly distinguishable. The two sexes or mating types are known as A and a. One can only tell the sex of an individual strain by putting it with another individual strain of a known sex for observation. If nothing happens they are of the same sex. If fusion takes place and ripe fruiting bodies are produced, they are of opposite sex. These fruiting bodies are called perithecia and contain the sexual or ascospores. These ascospores are formed in asci within the perithecium, each asci containing eight spores, each set of eight being the descendants of one nucleus. These eight spores carry the genetic characters of the two parent strains in a one to one ratio.

In addition to a sexual reproductive cycle, Neurospora multiplies profusely by vegetative means. Normal strains produce millions of microscopic orange spores called conidia which float about in the air and multiply the mold wherever conditions are favorable for growth. They multiply the individual without genetic change.

Neurospora grows readily in pure culture on a relatively simple medium. Its basic requirements for growth and reproduction are (1) a source of energy and carbon such as sucrose, (2) a suitable source of nitrogen, such as nitrates or ammonium salts, (3) inorganic salts supplying phosphate, sulfur, potassium, iron, calcium and other elements, and (4) biotin, a vitamin of the B-group. From these basic substances Neurospora is capable of synthesizing the remainder of the B-group vitamins, some 20 essential amino acids, nucleic acids containing various purines and pyrimidines, and certain yellow pigments which are precursors of vitamin A in higher animals.

Experimental Design

The Neurospora samples were supplied in two forms by the Biology Department of Stanford University. Conidia (asexual spores) were prepared in suspensions of horse serum, lyophilized and sealed in glass ampoules. Ascospores (sexual spores) from crosses of the

two mating types were mixed with Kaolin, dried and sealed in glass ampoules.

At Bikini on the USS BURLESON these ampoules of spores were wrapped in absorbent cotton and packed in cardboard mailing tubes. In this condition they were placed on the various target vessels selected for this purpose (3). During Test ABLE both conidia and ascospores were exposed while for Test BAKER only ascospores were used. Following Test ABLE the samples were collected within the first two days and placed in a refrigerator aboard the USS BURLESON. It was six days after Test BAKER before all samples could be recovered due to the radioactive contamination of the target vessels. The mailing tubes containing the samples were also contaminated. These containers were discarded and the samples also refrigerated. Upon return of the USS BURLESON to the United States all Neurospora samples were delivered to the Division of Biology, California Institute of Technology, Pasadena.

Results

The Test ABLE samples received primarily gamma radiation emitted at the time of explosion of the bomb. A few specimens on nearby ships received in addition some neutron irradiation. The Test BAKER samples, however, received only part of their irradiation at the time of the bomb explosion and were afterwards exposed to an unknown amount of gamma irradiation from contaminating fission products.

Study of the Neurospora samples was started in early summer of 1947. Lyophilized conidia (Samples 101-150), exposed during Test ABLE were crossed with wild type strains of the opposite sex. After ripe perithecia had formed several hundred random ascospores were dissected out and planted in individual tubes of complete agar medium. Each individual strain was then studied for mutant characters by the methods of Beadle and his associates. The results of this study are summarized in Table 1. The ascospores exposed during Test ABLE including controls (Samples 1-15 and 21-75) were no longer viable when the studies were started.

The ascospores exposed during Test BAKER (Samples 201-250) were suspended in sterile liquid minimal media (1,2) and heated to 60°C. for a period of 30 minutes. This is done in order to activate the spores and also has the advantage of killing any contaminating conidia. The suspension was then spread thinly on complete agar media plates so that the individual spores were well separated. After incubation one hyphal tip was picked from each discrete germinating spore and transferred to a tube of complete agar medium. The resulting strains of Neurospora which grew in 48 hours were then examined for mutant characteristics. These studies are summarized in Table 2.

Table 3 lists the biochemical mutants found during the course of this study. No attempt was made to classify the various morphological mutants.

Discussion

A comparison of the types of biochemical mutants found among the descendants of Bikini-exposed material with those previously recovered following treatment with X-ray, ultraviolet and mustard gas (2,4,5) indicates the atomic bomb ionizing radiation induces many of the same categories of mutant changes. A detailed comparison cannot be made until all the mutants recovered are completely identified both genetically and biochemically. In the course of future work this will be done for many of them, but a complete and systematic characterization of all of them is such a laborious and time consuming task that it is probably not worth while.

Of the 80 biochemical mutant types recovered in the Bikini material, 32 are classified as "unknowns". This means that these mutants require for growth, compounds that are either not yet known or that are not yet identified. This appears to be a high proportion of unknowns as compared with that found in previous tests with controlled X-ray and ultraviolet radiation. It should be pointed out, however, that previous experience shows that as mutants in the "unknown" group are studied more thoroughly many of them are transferred to the "known" category. In some instances growth requirements are obscured by inhibiting substances present in the test media. Occasionally mutant strains have double growth factor requirements. This may be a result of the presence of two mutant genes, or it may be the consequence of lack of ability of the strain to synthesize an intermediate substance common to two growth factors. These observations lead one to speculate as to whether exposure to the combination of gamma rays, fast and slow neutrons might produce a greater incidence of "unknown" mutant types than does X-ray or ultraviolet alone. Zirkle (6) has demonstrated that an additive lethal effect does occur in mice exposed to gamma rays and fast neutrons.

The numbers of recovered mutants are so small that no attempt has been made to correlate them with dosages measured by physical means at the various stations. For this type of study, maize is clearly a more favorable material.

Conclusion

The Bikini biochemical mutants constitute a valuable addition to the pool of genetic material found in previous experiments and available for biochemical and nutritional studies in Neurospora. Work on these mutant strains has already led to a significant increase in our knowledge of the ways in which organisms synthesize and metabolize vitamins, amino acids, purines, pyrimidines and other essential constituents of protoplasm. It is becoming increasingly clear that many of the processes of protoplasmic metabolism are basically the same in all forms of life. For example, it appears that the ornithine cycle is essentially the same in Neurospora and man. Neurospora transforms the amino acid tryptophane into nicotinic acid. The rat, and presumably also man, is able to make this conversion by the same mechanism.

The opportunities for increasing our knowledge of living systems through the use of induced gene changes are very great - we have only begun to exploit them.

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Table 1

Mutants Isolated from Conidia Exposed During Test ABLE

Sample No.	Estimated Dose r. Units	Number Spores Isolated	Number Grew	Percent Grew	Morph. Mutants	Biochem. Mutants	Total Mutants	Percent Mutants
101	7,400	482	452	91.8	1	2	3	0.6
102	8,800	616	517	83.9	6	2	8	1.5
105	12,700	318	242	76.1	2	0	2	0.8
107	3,050	420	375	89.2	1	0	1	0.2
108	3,000	200	188	94.0	1	0	1	0.5
109	391	428	394	92.0	4	2	6	1.5
110	729	200	177	88.5	2	1	3	1.6
111	81	98	81	82.6	1	1	2	2.4
112	107	256	237	92.5	4	0	4	1.6
113	62	619	571	92.2	3	0	3	0.5
114	62	576	468	81.2	6	4	10	2.1
115	70	364	302	82.9	3	0	3	0.9
116	132	881	725	82.2	7	1	8	1.1
117	20	451	360	79.8	2	0	2	0.5
118	38	457	400	87.5	5	3	8	2.0
119	6	629	537	85.3	3	1	4	0.7
120	38	599	525	87.6	3	4	7	1.3
121	0	300	247	82.3	0	0	0	0
122	0	1048	920	87.7	0	0	0	0
123	2	802	630	78.5	0	0	0	0
124	2	1002	858	85.6	0	0	0	0
125	18	1040	902	86.7	0	0	0	0
126	24	1020	860	84.3	2	0	2	0.2
127	0	906	741	81.7	1	0	1	0.1
128	1	1100	984	89.4	0	0	0	0
131	6	1048	870	83.0	1	0	1	0.1
132	11	1098	888	80.8	2	1	3	0.3
133	0	1069	848	79.3	0	0	0	0

Table 1 Cont'd

Mutants Isolated from Conidia Exposed During Test ABLE

Sample No.	Estimated Dose r. Units.	Number Spores Isolated	Number Grew	Percent Grew	Morph. Mutants	Biochem. Mutants	Total Mutants	Percent Mutants
134	0	912	755	82.7	0	0	0	0
135	2	1065	880	82.6	2	0	2	0.2
136	2	1091	870	79.7	0	0	0	0
137	0	701	577	82.3	0	0	0	0
138	0	806	632	78.4	1	0	1	0.1
139	0.2	665	569	85.5	1	0	1	0.1
140	2	1024	841	82.1	3	0	3	0.3
141	0	1076	916	85.1	0	1	1	0.1
142	0	1055	879	83.3	0	0	0	0
143	0	1129	822	72.8	0	1	1	0.1
144	0	1112	721	64.8	1	0	1	0.1
Controls		200	171	85.5	0	0	0	0

Table 2

Mutations from Hyphal Tips Isolated from Ascospores Exposed During Test BAKER

Sample No.	Estimated Dose r. Units	Number Tips Isolated	Number Tips Grew	Percent Grew	Morph. Mutants	Biochem. Mutants	Total Mutants	Percent Mutants
201	5	1100	975	88.6	0	0	0	0
209	7000	1216	804	66.1	22	15	37	4.6
210	7000	1094	623	56.9	0	6	6	0.9
213	10	682	558	81.8	0	0	0	0
214	10	751	638	84.9	3	0	3	0.4
217	1750	1244	1037	83.3	0	0	0	0
221	1	1375	1169	85.0	1	0	1	0.08
229	3	1350	1144	84.7	0	1	1	0.08
233	9000	2400	2117	88.2	0	18	18	0.85
234	9000	3000	2841	94.7	41	22	63	2.2
239	3500	1200	1025	85.4	1	0	1	0.09
Controls		500	485	97.0	0	0	0	0

Table 3

Biochemical Mutants of *Neurospora crassa* Isolated From Samples Exposed During the Bikini Atomic Bomb Tests.

	Number Found
Arginineless	1
Asparticless	1
Casein Unknown	8
Casein, Vitamin Unknown	2
Casein, Yeast Unknown	3
Cholineless	2
Cytidineless	2
* Cytidineless, Ribonucleic Acidless	7
Cystineless	1
l-Leucineless	2
l-Lysineless	2
Methionineless	7
Nicotinicless	1
Para-aminobenzoic Acidless	1
* Para-aminobenzoic Acidless, Folic Acidless	1
* Para-aminobenzoic Acidless, Cholineless	1
Serineless	1
Succinic Acidless	1
Thiamineless	3
Vitamin Unknown	2
Yeast Extract Unknown	19
Yeast Nucleic Acidless	12
Total	80

* Will grow on either compound

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